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Effects of Glutamic Acid, Lysine and Certain Inorganic Ions on Bovine Alkaline Phosphatases

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Introduction

Anions (borate, phosphate, arsenate) inhibit the alkaline phosphatase from bovine intestinal mucosa more than the phosphatase from milk, whereas the reverse is true of the cation ethanolamine (1). Inhibition of both enzymes by anions is competitive with the substrate; that of cations is noncompetitive (1). Glutamic acid inhibits the alkaline phosphatase from rat intestine more than the phosphatases from bone and kidney; the reverse is true of the basic amino acids lysine and histidine (2).

It has been suggested (1) that the relative effects of anions and cations might distinguish two types of alkaline phosphatase. With this in mind, the effects of glutamic acid and lysine, as well as carbonate and ammonium ions, on the bovine phosphatases have been investigated. The type of inhibition exerted by the amino acids has also been determined.

Experimental Methods

Preparation of the Phosphatases

Preparation of the milk enzyme has been described (1). The stock solution contained 0.14% protein, and when assayed in a dilution of 1:25 gave a reading of 12.0.

The stock solution of the intestinal mucosa enzyme (1, 3) contained 10 mg./100 ml., and when assayed in a dilution of 1:25 gave a reading of 32.0.

Assay of Alkaline Phosphatase (1)

The assay was performed in 5 min. at 37° in a volume of 12.0 ml., with 0.0025 *M* magnesium chloride and with 0.00075 *M* phenyl phosphate as the substrate. The

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phenol released was determined colorimetrically. The results are reported as photometer readings (logarithmic scale) or as moles of phenol.

To lessen interference on the part of the buffers, the experiments with glutamic acid were performed in ethanolamine at pH 9.6, the optimum for the mucosa enzyme, and at pH 9.9, the optimum for the milk enzyme (1), and the experiments with lysine were performed in carbonate. The optimum pH for the milk enzyme in the carbonate buffer used is 9.5. An optimum of 9.6 in 0.21 *M* carbonate buffer has been reported (4). The mucosa enzyme in the carbonate buffer has an optimum pH of 9.3.

Results

Glutamic Acid

Figure 1 shows the inhibitory effect of glutamic acid on the mucosa phosphatase at pH 9.67. The reciprocals of the photometer readings

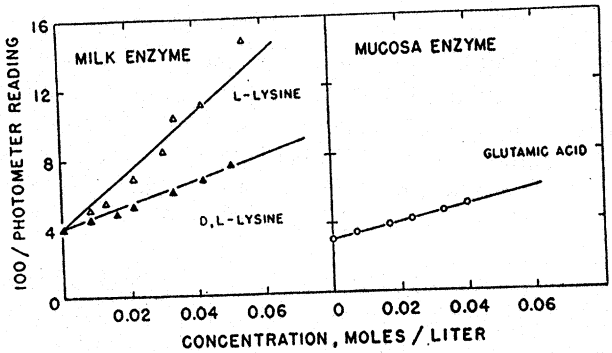

Fig. 1. Effect of glutamic acid on mucosa phosphatase (1:25) in 0.068 *M* ethanolamine, pH 9.67, and effect of lysine on milk phosphatase (2:25) in 0.068 *M* carbonate, pH 9.51.

are plotted against concentration of inhibitor. By extrapolation, a concentration of 0.070 *M* would give 50% inhibition. Glutamic acid was not inhibitory to the milk enzyme at either pH 9.6 or 9.9. A concentration of 0.040 *M* gave a 17.0% average increase in activity, but a concentration of 0.060 *M* produced only 15.0% increase.

Figure 2 shows the effect of 0.032 *M* glutamic acid on the mucosa enzyme with several concentrations of substrate. Comparison of these data with the results of similar experiments without glutamic acid shows that the inhibition was both competitive and noncompetitive (1). Without glutamic acid, the enzyme–substrate constant K_s was 0.00015 *M*; with glutamic acid, the *apparent* K_s was 0.00019 *M*. The

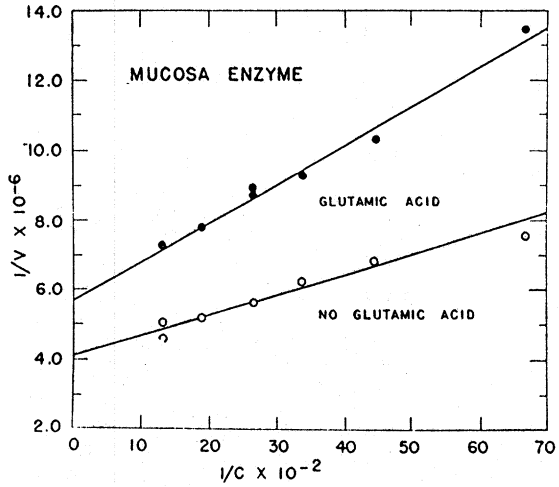

FIG. 2. Effect of glutamic acid on mucosa phosphatase (1:25) in 0.071 *M* ethanolamine, pH 9.67, in relation to the concentration (*C*) of the substrate phenyl phosphate.

maximum velocity ($V_{max.}$ from $1/V$ intercept) decreased from 2.47 to 1.76×10^{-7} mol./5 min.

Lysine

Figure 1 shows the effect of lysine on the milk phosphatase at pH 9.5. A reagent and inactive-enzyme blank were included with every assay. Blank values were especially high with lysine, which may explain the less regular data obtained with lysine (Figs. 1 and 3). A preparation of DL-lysine was one-half as inhibitory as a preparation of L-lysine, the 50% inhibition concentrations being 0.062 and 0.027 *M*, respectively. Comparison of DL-alanine and L-alanine showed that they were equally inhibitory with both milk and mucosa phosphatases.

Lysine was not inhibitory to mucosa phosphatase at either pH 9.3 or 9.5; 0.021 and 0.042 *M* of L-lysine had a negligible effect.

Figure 3 shows the effect of lysine on milk phosphatase in carbonate with several concentrations of substrate. Experiments are included for the enzyme in carbonate without lysine. The enzyme–substrate constant K_s in carbonate buffer was 0.00039 *M* and the $V_{max.}$ was 3.6×10^{-7} mol./5 min. Analysis of the lysine experiments at *high* concentra-

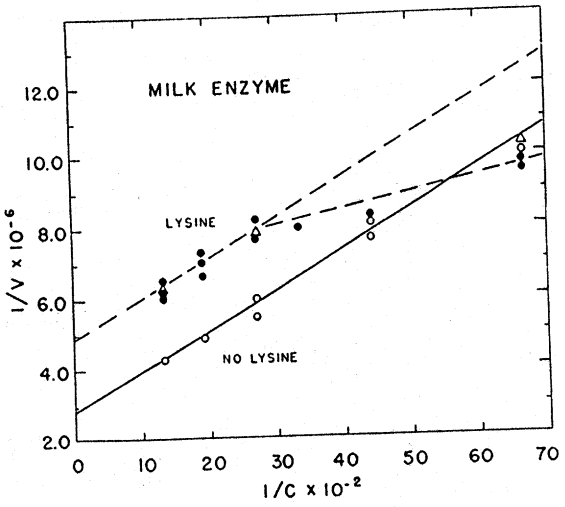

FIG. 3. Effect of lysine on milk phosphatase (2:25) in 0.071 *M* carbonate, pH 9.51, in relation to the concentration (*C*) of the substrate phenyl phosphate. ○, no lysine; ●, 0.033 *M* DL-lysine; △, 0.017 *M* L-lysine.

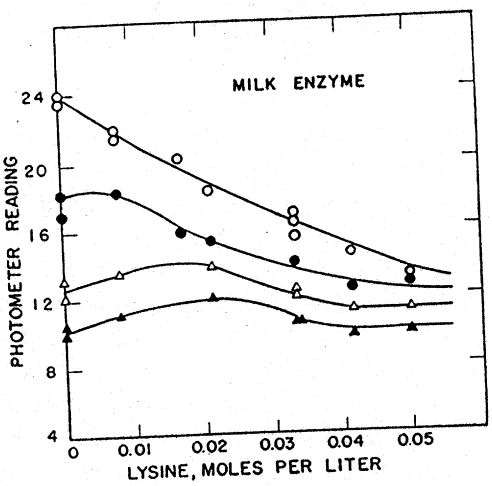

FIG. 4. Effect of DL-lysine on milk phosphatase (2:25) in carbonate, pH 9.51, with different concentrations of the substrate phenyl phosphate. ○, 0.00075 *M* substrate; ●, 0.00038 *M*; △, 0.00023 *M*; ▲, 0.00015 *M*.

tions of substrate led to about the same K_s value, but the $V_{max.}$ had decreased to 2.1×10^{-7} mol./5 min. This is characteristic of noncompetitive inhibition. It is not certain, however, that some competitive inhibition was not masked by the stimulation which lysine began to show at low concentrations of substrate. DL- and L-lysine were alike in showing this effect (Fig. 3). Figure 4 shows the results obtained with other concentrations of lysine and several substrate concentrations.

Carbonate

The activity of the mucosa and milk phosphatases was determined in 0.068, 0.034, and 0.017 *M* carbonate–bicarbonate buffers with the pH carefully controlled at 9.51. The respective assay readings in these buffers were: for milk enzyme (2:25) 23.7, 25.2, and 26.6; for mucosa enzyme (1:25) 15.5, 19.0, and 20.0. The data obtained were plotted in the manner shown in Fig. 1. The concentration causing 50% inhibition of the milk enzyme was 0.47 *M*; the concentration producing the same inhibition of the mucosa enzyme was 0.15 *M*. In earlier experiments, in which carbonate was added to the mucosa enzyme in ethanolamine buffer, a value of 0.21 *M* was obtained (1).

Ammonium Ion

Ammonium hydroxide–ammonium chloride buffer of 0.068, 0.034, and 0.017 *M* final concentrations was used. Assays were performed at three pH values. The experiments with the mucosa enzyme were in the range pH 9.3–.5. The optimum was above pH 9.5. All values fell on the same curve; hence it can be concluded that the ammonium ion exerts little inhibition on this enzyme.

Experiments with the milk enzyme (2:25) were performed in about the same pH range, and here too the optimum was above pH 9.5. Curves through the assay values were parallel, and at pH 9.5 the readings for the different buffer concentrations were 20.6, 22.8, and 23.8, respectively. The value giving 50% inhibition was calculated to be 0.34 *M*.

Discussion

Table 1 shows the inhibition patterns of the bovine alkaline phosphatases revealed by the present studies, together with data obtained

by Bodansky (2) and Aebi (10, 11) with other phosphatases. The concentration of glutamic acid (0.070 *M*) giving 50% inhibition of the bovine intestinal mucosa phosphatase was higher than that found by Bodansky (2) (0.030 *M*) for rat intestinal phosphatase with glycerophosphate as the substrate. There may be a species difference in the two enzymes, but the difference in the inhibition was in the direction expected for a high (glycerophosphate) and a low (phenyl phosphate) enzyme–substrate dissociation constant (K_s).

TABLE I

Inhibition Pattern of Alkaline Phosphatases

Enzyme	L-Glutamic acid		L-Lysine		Carbonate[a]		Ammonium ion[b]	
	50% inhibition conc.	Type of inhibition	50% inhibition conc.	Type of inhibition	50% inhibition conc.	Type of inhibition	50% inhibition conc.	Type of inhibition
Bovine intestinal mucosa	0.070 *M*	Largely noncompet.	No effect[c]	—	0.15 *M*	—	No effect	—
Bovine milk	Stimulation	—	0.027 *M*	Noncompet.	0.47 *M*	—	0.34 *M*	—
Rat intestine (2)	0.030 *M*	Noncompet.	0.06–0.14 *M*	—	—	—	—	—
Rat kidney and bone (2)	0.1 *M*	—	0.01 *M*	—	—	—	—	—
Rat and horse kidney (10, 11)	0.6 *M*[d]	—	0.048 *M*[d]	—	0.25 *M*[e] 0.22 *M*[d]	Compet.	0.15, 0.17 *M*[d]	Noncompet.

[a] Tetraborate, studied previously (1), is more inhibitory than carbonate, but shows about the same relative inhibition for the two bovine phosphatases. The inhibition with both enzymes is competitive.

[b] Ethanolamine is more inhibitory than the ammonium ion, but the inhibition pattern is the same (1).

[c] The term "no effect" is relative, but the maximum concentrations of the inhibitors studied did not exceed 0.1 *M*.

[d] Calculated from data in Ref. (11).

[e] Calculated from data for the 0.015 *M* concentration of the substrate glycerophosphate in Ref. (10).

The relative proportion of the two types of inhibition exerted by glutamic acid was calculated in the manner employed by Bodansky for the effect of glycine on intestinal phosphatase (5). Inhibition by glutamic acid is 100% noncompetitive in an infinitely concentrated substrate solution, and 96% noncompetitive in 0.0020 *M*, decreasing to 74% in 0.00014 *M* phenyl phosphate. The competitive inhibition did not exceed the effect of glycine on rat intestinal phosphatase (5) and glycine lacks an extra free-carboxyl-group. Bodansky, who studied the

effect of glutamic acid on rat intestinal phosphatase with high concentrations of glycerophosphate (0.0127 to 0.0254 *M*), observed only noncompetitive inhibition (2). Thus although glutamic acid resembles inorganic anions in being more inhibitory to intestinal mucosa than to milk phosphatase, it is impossible to say whether it is inhibitory because it is an anion, for the inhibition pattern resembles that shown by glycine (5). Inorganic ions (borate, phosphate, and carbonate), on the other hand, are almost exclusively competitive inhibitors (1).

Bodansky (2) found that glutamic acid was inhibitory to rat bone and kidney phosphatases (50% inhibition at about 0.10 *M*), the group to which milk phosphatase may belong. A close comparison of the stimulation shown by the milk phosphatase cannot be made because of species and substrate differences. Bodansky (5) employed 0.006 *M* glycine in his assays for maximum activity; however, the time of hydrolysis exceeded 5 min., and activation was not observed in experiments of 5-min. duration (6). Glycine and alanine gave no activation in the system used in the present studies.

Bodansky (2) found that both lysine and histidine were more inhibitory to rat intestinal phosphatase than to bone and kidney phosphatases. Histidine was much more inhibitory (concentrations giving 50% inhibition were 0.003–0.006 *M*) than other amino acids, and its strong affinity for metals might put it in a special category; hence the present studies were limited to lysine as representative of a simpler type of cation.

The apparent optical specificity shown by lysine in its inhibition of milk phosphatase has not been reported for inhibition of phosphatase by other amino acids. Only L-α-amino acids are inhibitory to arginase (7), which is understandable for the competitive lysine and ornithine, but it is less understandable for amino acids that are noncompetitive inhibitors.

Lysine is more inhibitory to mucosa than to milk phosphatase, as are the cations ethanolamine (1) and ammonium ion. The noncompetitive inhibition in concentrated solutions of the substrate is the same type as that shown by cations and also the type shown by lysine to the rat phosphatases (2). This is also the type of inhibition shown by other amino acids (2, 5). The shift from inhibition to stimulation of the milk phosphatase by lysine at low concentrations of substrate has not been observed for any other inhibitors of alkaline phosphatase. The change

in the effect of lysine may be related to the fact that the optimum pH of alkaline phosphatase shifts to lower values with decreasing concentrations of substrate (8) and accordingly experiments at low substrate concentrations were above the pH optimum. Support for this explanation was obtained by experiments above the pH optimum for the highest concentrations of substrate (0.00075 *M*) employed in the experiments presented in Fig. 4. At pH 9.66, the effect of lysine was that shown by the curve for 0.00038 *M*, that is, low concentrations of lysine stimulated the enzyme. At pH 9.35, the lysine had the same effect as at the pH optimum (pH 9.51), that is, inhibition at all concentrations of lysine.

The carbonate anion was more inhibitory to the mucosa than to the milk phosphatase, but the reverse was true for the ammonium cation, which followed the pattern previously observed for inorganic ions (1). Carbonate and ammonium buffers were about equally inhibitory to milk phosphatase and to kidney phosphatase, but the mucosa phosphatase was more inhibited by the carbonate buffer. Some reports (9) have given the impression that ammonium ion is more inhibitory than carbonate ion. Carbonate is more inhibitory to the kidney phosphatase (11) than it is to the milk phosphatase, but this would be expected of a competitive inhibitor with substrates of the different K_s values shown by glycero- and phenyl phosphates. However, the noncompetitive ammonium ion showed about the same relative difference.

Summary

Glutamic acid was more inhibitory to the alkaline phosphatase of bovine intestinal mucosa alkaline than to milk phosphatase; the reverse was true of lysine. Glutamic acid inhibition was predominantly noncompetitive; the inhibition pattern resembled that of glycine rather than the competitive inhibition shown by inorganic anions. Lysine activated milk phosphatase at low substrate concentrations.

Carbonate ion was more inhibitory to mucosa phosphatase than to milk phosphatase; the reverse was true of inhibition by the ammonium ion.

The over-all inhibition pattern suggests that there are two types of alkaline phosphatases. One is the intestinal enzyme; the milk enzyme, which is similar to the bone and kidney phosphatases, is the other.

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